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Title of invention

"Substances binding zoonotic Helicobacter species and use thereof"
(Zoonoottisia Helicobacter-lajeja sitovia aineita ja niiden käyttö)

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Substances binding zoonotic *Helicobacter* species and use thereof

FIELD OF THE INVENTION

The present invention is related to carbohydrate binding specific zoonotic and enterohepatic *Helicobacter* species causing hepatobiliary and/or enteric diseases including diarrhea. Furthermore the invention is related to gastric diseases causing zoonotic *Helicobacter* species. The group is abbreviated *zHelicobacter* (zoonotic *Helicobacter* species). This group of *Helicobacter* does not include species specific animal or human pathogens which are causing solely gastric diseases such as *Helicobacter mustelae* or human specific *Helicobacter pylori*. The *zHelicobacteria* are infecting both human and pet animals of human and have zoonotic capacity to infect humans, especially persons with weak immune system. The present invention characterizes the carbohydrate binding specificities of *zHelicobacter* which are able to mediate the cross-species infective actions of the bacteria.

BACKROUND OF THE INVENTION

There are more than 20 characterised *Helicobacter* species to date (On, 2001). The species have been isolated from several hosts including primates, pigs, felines, canines, poultry and rodents (On, 2001). In their hosts, *Helicobacter* spp. have been identified from both the gastric and enterohepatic niches of the gastrointestinal tract, where they have been associated with a wide spectrum of clinical outcomes (Fox *et al.*, 2000; Nilsson *et al.*, 2001).

Carbohydrates binding to the human gastric pathogen *H. pylori*

Helicobacter pylori is the most widely studied species of the genus and is associated with gastric pathology (Hunt 1996). In particular the bacterium has the noted ability to attach to both Lewis^b (Le^b) (Borén *et al.*, 1993), and Sialyl-dimeric-Le^x antigens which may be extremely relevant in the maintenance of a chronic infection (Gerhard *et al.*, 2001; Madhavi *et al.*, 2002). Glycoconjugates, both lipid- and protein-based, have been reported to serve as receptors for the binding of this microorganism as, *e.g.*, sialylated glycoconjugates (Evans *et al.*, 1988), sulfatide and GM3 (Saitoh *et al.*, 1991), polyglycosylceramides (Miller-Podraza *et al.*, 1996; 1997a), lactosylceramide (Ångström *et al.*, 1998) and gangliotetraosylceramide (Lingwood *et*

al., 1992; Ångström *et al.*, 1998). Other potential receptors for *Helicobacter pylori* include the polysaccharide heparan sulphate (Ascensio *et al.*, 1993) as well as the phospholipid phosphatidylethanolamine (Lingwood *et al.*, 1992). Binding to lactotetraosylceramide (Teneberg, *et al.*, 2002) and to type 2 lactosamines (PCT/FI02/00043) has been recently described.

US patents of Zopf *et al.*: 5,883,079 (March 1999), 5,753,630 (May 1998) and 5,514,660 (May, 1996) describe Neu5Acα3Gal- containing compounds as inhibitors of the *H. pylori* adhesion. The sialyl-lactose molecule inhibits *Helicobacter pylori* binding to human gastrointestinal cell lines (Simon *et al.*, 1997) and is also effective in a rhesus monkey animal model of the infection (Mysore *et al.*, 1999). The compound is in clinical trials. US patent Krivan *et al.* 5,446,681 (November 1995) describes bacterium receptor antibiotic conjugates comprising an asialo ganglioside coupled to a penicillin antibiotic. Especially is claimed the treatment of *Helicobacter pylori* with the amoxicillin-asialo-GM1 conjugate. The oligosaccharide sequences/glycolipids described in the invention do not belong to the ganglioseries of glycolipids. US patents of Krivan *et al.*: 5,386,027 (January 1995) and 5,217,715 (June 1993) describe the use of oligosaccharide sequences or glycolipids to inhibit several pathogenic bacteria but *Helicobacter* species according to the invention were not shown.

The references above list carbohydrate receptors of *H. pylori*, which is not the target of the present invention. The invention is further directed to the treatment of enteric diseases especially diarrhea, and hepatobiliary diseases including gall bladder stones and liver cancers.

It has been established previously that both *H. pylori* and *H. mustelae* bind ganglio-tetraosylceramide which was confirmed in this study (XXXXMilh et al). The species are not among the *zHelicobacter* species according to invention but were tested as control species.

Binding specificities of E. coli

Human and animal diarrheas have been studied with different pathogens such as various types of *Escherichia coli*. These studies do not include *zHelicobacter* species. Galβ3GlcNAc or Galβ4GlcNAc usage was also patented. It was suggested that sialic acid may be necessary for EPEC mediated cell detachment (Vanmale, R.P. *et al.*, 1995). In another study the same scientist inhibited attachment of an EPEC-strain to

Hep-2 cells by N-acetyl lactosamine-BSA and Lex –BSA neoglycoproteins in the concentration range 0.4- 0.8 mg/ml (Vanmale, R.P. *et al.*, 1999).

An EPEC strain was shown to bind in decreasing order of activity asialo-GM1, asialo-GM2, globoside and lacto-N-tetraose were observed to bind, while sialylated gangliosides, lactosylceramide, globotriaosylceramide (Galα4Galβ4GlcβCer), and Forssmann glycolipid were negative. Asialo-GM1 binding was studied with several strains. The binding active epitope was considered to be GalNAcβ4Gal or GalNAcβ3Gal with weaker activity. The authors also describe binding to asialo-GM1 neoglycoprotein and GalNAc neoglycoprotein but not inhibition of the binding to the asialo-GM1 by neoglycoproteins at 25 micromolar concentration or undefined oligosaccharides at 1 mM concentration (Jagannatha, H.M. *et al.*, 1991). Their results indicated specifically that the contradictory bindings described were not inhibitable by monovalent or polyvalent oligosaccharide sequences and therefore this study did not show therapeutically useful types of binding as the present invention does.

Several oligosaccharide fractions from human milk were analysed for inhibition of EPEC strains at a concentration 3 mg/ml. Inhibiting activity was observed in pentasaccharide fraction, possible difucosylactose fraction, possible lacto- and neolactotetraose fraction, heptasaccharide fraction and hexasaccharide fraction. The fractions were named after expected major components. The real compositions of the fractions and the presence of potential minor or other saccharides were not assessed (Cravioto, A., *et al* 1991).

Human milk lactoferrin, secretory IgA and free secretory component have been shown to inhibit EPEC-binding to glycoproteins of HELA-cells, with no indications to carbohydrate structures (Nascimento de Araújo and Giugliano 2001).

Inhibition of the EHEC toxin binding to Galα4Galβ4Glc and binding data about other toxins of *E.coli* binding to Galβ4GlcNAcβ3Galβ4Glc has not been shown to cure the disease caused by EHEC. There are suggestions with regard to the use of solid phase conjugates containing Galα4Galβ4Glc for inhibition of toxins in therapeutics against diarrhea. The clinical trials using the single epitopes failed. The polyvalent conjugates

according to the present invention are specifically directed to soluble polyvalent conjugates for effective inhibitions of pathogens, especially adhesion of diarrhea causing *E. coli* bacteria.

Purified colonialization factors of certain ETEC strains were shown to bind to asialo-GM1 (Galβ3GalNAcβ4Lac-Cer) but not to sialylated control gangliosides (Oroe *et al.*, 1990). A colonialization factor was shown to bind to several galactoglycoproteins in the rabbit intestine. This binding could be inhibited by asialo-GM1, GM1, GM2, but not so effectively by GM3 and the adhesin bound to GalNAcβ4Gal-neoglycoprotein. Human meconium glycoprotein and its asialo- and afucoform inhibited the binding more weakly and bovine glycophorin most weakly. As the binding of the *Maackia amuriensis* lectin, the meconium glycoprotein binding was also probably polylactosamine dependent. Sialic acid residues were considered not to be important for the bindings (Neeser, J.R. *et al*, 1989; Wennerås, C. *et al.* 1995). This study shows no useful defined multiepitope solution for treatment of diarrheas or other infections. The polylactosamine specificity was not defined, if present. The present invention shows that not all of polylactosamine type sequences, such as the branched structure, are not active. Use of combinations of specificities are not defined.

Human milk gangliosides GM1 and GM3 and more weakly GD3 were inhibiting the binding of an ETEC and an EPEC strain to human cancer Caco-2 cells, while lactosylceramide, GD3-lactone, and N-acetylneuraminic acid was negative. The present invention shows a lactosylceramide binding and sialic acid dependent bindings. This prior art shows a potential single not well characterized specificity which, if existant, is probably not even among the binding specificities disclosed in the present invention.

Uncharacterized possibly sialic acid related binding has been reported from ETEC strains (Barthus *et al*, 1985; Evans *et al*, 1979; Pieroni, P. and Worobec, E.A. 1988, Wennerås *et al.*, 1990).

SUMMARY OF THE INVENTION

The present invention is directed to the use of a galactoseβ3/4-based binding epitope for *zHelicobacter* species comprising an oligosaccharide sequence as defined below. The invention is also directed to therapeutical compositions comprising at least one pathogen inhibiting oligosaccharide sequence selected from the groups of pathogen receptors as defined below.

The present invention is directed to *non-H.pylori Helicobacter* species, especially to enterohepatically infecting ones causing diarrheas and liver diseases. Typically these bacteria, referred as *zHelicobacter* (*zHelicobacteria* in plural), are zoonotically active infecting both human and animals, such as cattle and pets, preferred pet animals are cats and dogs. In a separate embodiment the present invention is directed to gastric infections caused by *zHelicobacteria*. The prior art is directed to different species of gastric bacteria such as *H. pylori*, *H.mustelae* (a non-zoonotic gastric pathogen of ferrets), and various non-*Helicobacter* species infecting the intestinal tract such as various types of *Escherichia coli* causing diarrheas. Different species of bacteria have different binding specificities and the receptors of *zHelicobacteria* are not known from prior art. Especially big differences could be expected between bacteria infecting different localizations in gastrointestinal tract or belonging to totally different families such as *Helicobacter* and *E. coli*. The present invention revealed different binding specificity profiles between *zHelicobacter* and *H. pylori*. The zoonotic bacteria reveals a specific group of receptors of zoonotic bacteria.

The invention further describes a simultaneous use of at least two carbohydrate receptors of the above groups binding to pathogens, especially zoonotic *Helicobacter* species, *zHelicobacter*, and analogs or derivatives of the oligosaccharide sequence having binding activity to *zHelicobacter*, for the treatment and prophylaxis of diseases, especially diarrheas, due to the presence of *zHelicobacter*.

Among the objects of the invention are the use of the diarrheagenic *zHelicobacter* binding oligosaccharide sequences described in the invention as a medicament, and the use of the same for the manufacture of a pharmaceutical composition, particularly for the treatment of any condition due to the presence of *zHelicobacter*.

The present invention also relates to the methods of treatment for conditions due to the presence of diarrheagenic *zHelicobacter*. The invention is also directed to the use

of the receptor(s) described in the invention as an *zHelicobacter*-binding or -inhibiting substance for diagnostics of *zHelicobacter* especially diarrheagenic *zHelicobacter*.

Another object of the invention is to provide substances, pharmaceutical compositions and nutritional additives or compositions containing *zHelicobacter*-binding oligosaccharide sequence(s).

Other objects of the invention are the use of the above-mentioned *zHelicobacter* binding substances for the typing of *zHelicobacter*, and the *zHelicobacter* binding assays.

The invention is also directed to the use of the oligosaccharide sequences according to the invention in food safety products for inhibition of pathogens, including disease causing and especially bacteria such as *zHelicobacter*. The present invention is also directed to food safety analytics to determine the presence of disease, especially diarrhea, causing *zHelicobacter* by the use of the receptor carbohydrates according to the invention.

A BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Comparison of glycosphingolipid recognition by (B) *Helicobacter hepaticusi* and (C) *Helicobacter bilis* using the chromatogram binding assay. The lanes were: 1, acid glycosphingolipid fraction of human granulocytes, 40 μg; 2, Galβ4Glcβ1Cer (lactosylceramide) of dog intestine, 2 μg; 3, Galα3Galβ4Glcβ1Cer (isoglobotriaosylceramide) of dog intestine, 2 μg; 4, Galβ3GalNAcβ4Galβ4Glcβ1Cer (gangliotetraosylceramide) of mouse feces, 2 μg; 5, Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer (Le^a-5 glycosphingolipid), 2 μg; 6, Fucα2Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer (Le^b-6 glycosphingolipid), 2 μg; 7, GalNAcβ3Galα4Galβ4Glcβ1Cer (globotetraosylceramide) human erythrocytes, 2 μg; 8, lactotetraosylceramide (Galβ4GlcNAcβ3Galβ4Glcβ1Cer), 2 μg.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is related to *non-H.pylori Helicobacter* species, especially to enterohepatically infecting ones causing diarrheas and liver diseases. Typically these bacteria, referred as *zHelicobacter* (*zHelicobacteria* in plural), are zoonotically active infecting both human and animals, such as cattle and pets, preferred pet animals are cats and dogs. In a separate embodiment the present invention is directed to the treatment of gastric infections caused by *zHelicobacteria*. The prior art is directed to different species of gastric bacteria such as *H. pylori*, *H. mustelae* (a non-zoonotic gastric pathogen of ferrets), and various non-*Helicobacter* species infecting the intestinal tract such as various types of *Escherichia coli* causing diarrheas. Different species of bacteria have different binding specificities and the receptors of *zHelicobacteria* are not known from prior art. Especially big differences could be expected between bacteria infecting different localizations in gastrointestinal tract or belonging to totally different families such as *Helicobacter* and *E. coli*. The present invention revealed different binding specificity profiles between *zHelicobacter* and *H. pylori*. The zoonotic bacteria reveal a specific group of receptors of zoonotic bacteria.

The group of *zHelicobacter* does not include species specific human *Helicobacter pylori*. The present invention is further not directed to the infection of ferrets by *H. mustelae* as this is not an infection of a pet animal or cattle with a risk of zoonosis due to contact with human. The *zHelicobacteria* are infecting human and/or, preferably and, pet animals of human and have zoonotic capacity to infect humans, especially persons with weak immune system. The present invention characterizes the carbohydrate binding specificities of *zHelicobacter* which are able to mediate the cross-species infective actions of the bacteria.

Overview of results

The inventors analysed binding specificities of several *zHelicobacter* species towards a library of glycolipids in a TLC-overlay assay.

It has been established previously that both *H. pylori* and *H. mustelae* bind gangliotetraosylceramide binding was demonstrated for *H. felis*, *H. canis* and *H. hepaticus* and *H. bilis* (Table 1). Furthermore, in common with *H. pylori* we found that both gastric and enterohepatic *Helicobacter* spp. tested were capable of binding to lactotetraosylceramide, lactosylceramide with phytosphingosine and/or hydroxy fatty

acids and isoglobotriaosylceramide. In contrast, binding to Le^b glycosphingolipid was only observed for *H. pylori* CCUG 17875 (Table 1).

The binding of certain *H. pylori* strains to slow-migrating gangliosides in the acid glycosphingolipid fraction of human granulocytes is sialic acid-dependent (Miller-Podraza *et al.*, 1999), and this fraction was therefore used as an indicator of sialic acid-recognition. The sialylated structures in human granulocytes are mainly NeuNAcα3Gal- and NeuNAcα6Gal. Binding to this fraction was noted for *H. hepaticus* CCUG 33637 (exemplified in Fig. 1B. lane 1) and *H. pylori* CCUG 17874 and occasionally for *H. mustelae* CCUG 25715 (Table 1). Sialic acid binding capacity assayed by other substances is also present in some species of *H. bilis*.

The *zHelicobacter* species were further observed to bind a linear polylactosamine glycolipid. The binding epitope is in the polylactosamine backbone as the removal of the specific terminal does not essentially effect the binding.

The present invention noticed that the carbohydrate specificities are also observable by various other methods in addition to the glycolipid assays. The binding were observable by assay involving protein type glycoconjugates even in cell based assay including traditional cell assay with cells from various species. These assays give results supporting the analysis of glycolipids.

Preferred carbohydrate structures to be used according to invention

β-Galactose based reseptors

According to the present invention the most common binding specificity of profile of the *zHelicobacter* species Galactoseβ3/4 -based receptor includes structures according to the formula:

$$[Gal\beta y]_p[Hex(NAc)_r\alpha z/\beta z]_sGal\beta x[Glc(NAc)_u]_v \qquad (I)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, and z is either linkage position 3 or 4, and Hex is either Gal, or Glc ,

so that

when v is 1 and u is 0 then x is 4,

when v is 0 then s is 1 and preferably also p is 1

when s is 0 the also p is 0 and v is 1

when p is 1, and y=3, Hex is Galβ or Glcβ and r=1, or p is 1 and y=4 and Hex is Glcβ and r=1 so that the terminal Gal is β3- or β4- linked to GlcNAcβ or the terminal Gal is β3-linked to GalNAcβ),

when p is 0 and z is 4, then Hex is Galβ and r is 1 so that the terminal monosaccharide structure is GalNAcβ4, or p =0 and z=3 so that the terminal is HexNAc/Hexα/β3),

when there is nonreducing terminal Galβ3/4, this can be further substituted by SAα3/6, wherein SA is a sialic acid, preferably NeuNAc, N-acetylneuraminic acid.

<u>β-Galactose based reseptors, a combination formula:</u>

Collectively the Galactoseβ3/4 -based receptors is an oligosaccharide sequence according to formula

$$[Gal\beta y]_p[Hex(NAc)_r\alpha z/\beta z]_sGal\beta x[Glc(NAc)_u]_v \qquad (II)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, and z is either linkage position 3 or 4 or 6, and Hex is either Gal, Glc or SA (sialic acid),

so that

when v is 1 and u is 0 then x is 4

when v is 0 then s is 1 and preferably also p is 1,

when s is 0 the also p is 0 and v is 1

when p is 1, and y=3, Hex is Galβ or Glcβ and r=1, or p is 1 and y=4 and Hex is Glcβ and r=1 (the terminal Gal is β3- or β4- linked to GlcNAcβ or the terminal Gal is β3-linked to GalNAcβ),

when Hex is SA, z is either 3 or 6, preferably 3,

when p is 0 and z is 4, then Hex is Galβ and r is 1(the terminal monosaccharide structure is GalNAcβ4), or p =0 and z=3 (the terminal is HexNAc/Hexα/β3), or

Hex is SA, z is 3 or 6 and the terminal structure is SAα3Gal or SAα6Gal.

In a preferred embodiment the Galβ-type receptor activity is a neutral oligosaccharide sequence not comprising sialic acid. In an embodiment the terminal p =0, Hex is sialic acid (SA), preferably, NeuNAc (N-acetylneuraminic acid) α3- or α6-linked.

Preferred neutral galactose based receptors according to the invention

According to the present invention the most common binding specificity profile of the *zHelicobacter* species Galactoseβ3/4 -based receptor includes structures according to the formula:

$$[Gal\beta y]_p[Hex(NAc)_r\alpha z/\beta z]_sGal\beta x[Glc(NAc)_u]_v \qquad (III)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, and z is either linkage position 3 or 4, and Hex is either Gal, or Glc ,

so that

when v is 1 and u is 0 then x is 4,

when v is 0 then s is 1 and preferably also p is 1

when s is 0 the also p is 0 and v is 1

when p is 1, and y=3, Hex is Galβ or Glcβ and r=1, or p is 1 and y=4 and Hex is Glcβ and r=1 so that the terminal Gal is β3- or β4- linked to GlcNAcβ or the terminal Gal is β3-linked to GalNAcβ),

when p is 0 and z is 4, then Hex is Galβ and r is 1 so that the terminal monosaccharide structure is GalNAcβ4, or p =0 and z=3 so that the terminal is HexNAc/Hexα/β3).

Major receptor types according to the invention

The formula above is further divided to major structure groups including

1. Lactose/lactosamine type carbohydrate receptor

   This group further includes Lactose- receptors, and lactosamine receptors including Lacto-receptors, and Neolacto receptors
2. Ganglio-receptors
3. Sialic acid receptor

Preferred lactose/lactosamine type receptors for zHelicobacter

$$[Gal\beta y]_p[Hex(NAc)_ra3]_sGal\beta x[Glc(NAc)_u]_v \qquad (IV)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, and a is either alpha or beta, and Hex is either Gal or Glc.

so that

when p is 1, Hex is Glcβ and r=1, and a is β (the terminal Gal is β3- or β4- linked to GlcNAcβ3)

when p is 0, then preferably

Hex is Gal, r is 0 and a is alpha (terminal structure is Galα3) or

Hex is Glc, r is 1 and a is beta (terminal structure is GlcNAcβ3)

In a preferred embodiment the lactose/lactosamine type receptors for zHelicobacter are according to the formula:

$$[Gal\beta y]_p[GlcNAc\beta 3]_s Gal\beta x[Glc(NAc)_u]_v \qquad (V)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, so that

at least p is 1 or v is 1,

when p is 1, s is 1

When u is 0, x is 4 and the reducing end Glc is preferably linked to hydroxyl.

Most preferred lactose/lactosamine structures include

the human milk tetrasaccharides Galβ4GlcNAcβ3Galβ4Glc and Galβ3GlcNAcβ3Galβ4Glc and lactosylceramides.

The preferred lactosamine structures also include

oligosaccharide sequences and oligosaccharides from the group Galβ4GlcNAc, Galβ3GlcNAc, Galβ4Glc, Galβ4GlcNAcβ3Gal, Galβ3GlcNAcβ3Gal, And GlcNAcβ3Galβ4Glc, GlcNAcβ3Galβ4GlcNAc, Galβ4GlcNAcβ3Galβ4GlcNAc, and Galβ3GlcNAcβ3Galβ4GlcNAc.

<u>The five receptor subgroups according to the invention</u>

a) Lactose receptors
b) Lacto-receptors
c) Neolacto-receptors
d) Ganglio-receptors
g) Sialic acid-receptors

The present invention is also directed to the use of the five receptor types in combination so that at least 2 receptors are used. It is also preferred to use any of the receptor subtypes together with another receptor type. It is preferred to use Lactose receptor together with lactosamine receptor and/or ganglio-receptor and/or sialic acid receptor. It is further preferred to use Lactose/lactosamine receptor together with a gangliore-ceptor and/or sialic acid receptor.

The present invention relates to a therapeutical composition comprising a purified fraction(s) of at least one, and in another embodiments of at least two or at least three compounds being or containing a pathogen inhibiting oligosaccharide sequence. When several oligosaccharide sequences are used, these are preferably selected from at least two, and in another embodiment from at least two, of the groups of pathogen receptors described above.

Lactose receptors

In broadest sense lactose receptors are structures comprising oligosaccharide sequence Galβ4Glc. In a preferred embodiment lactose receptors are lactosylceramide receptors wherein the lactose structure is linked to a ceramide. More preferably there is a hydroxyl fatty acid structure present on the ceramide. The present invention is especially directed to the use of lactose receptors especially lactosylceramides comprising hydroxy fatty acids against *zHelicobacter* infections.

The lactosylceramide receptors according to the present invention means a lactose residue comprising molecule in which lactosyl residue is linked to a ceramide structure comprising a natural type of hydroxylfatty acid or alternatively lactosylceramide receptor means a mimetic structure of lactosylceramide in which the lactosyl residue is linked to a hydroxyl group comprising a ceramide-mimicking structure. The hydroxyl group of the hydroxyl fatty acid or ceramide mimicking structure preferentially forms a hydrogen bond with Glc-residues linked to ceramide or ceramide-mimicking structure. The lactosylceramide or mimetic structure can be substituted at position 3 or 4 of the Gal residue by natural type oligosaccharide sequences. The lactosylceramide receptor glycolipids also includes lacto- and/or neolactoseries glycolipids comprising a hydroxyl fatty acid. In other embodiments the present invention is also directed to

the use of lacto- and/or neolacto- and/or ganglioseries glycolipids comprising a lactosyl residue and a hydroxylfatty acid. The present invention is also directed to the use of analogs of lacto- or neolactoseries oligosaccharide sequences linked to the hydroxyl group comprising ceramide-mimicking structure. The present invention is also directed to the use of analogs of ganglioseries oligosaccharide sequences linked to the hydroxyl group comprising ceramide-mimicking structure. In a preferred embodiment the invention is directed to the use of non-sialylated forms of lactosylceramide receptors according to the present invention. The preferred embodiments include molecules according to the following Formula

$R_1xGal\beta4Glc\beta R_2$ (VI)

wherein x is linkage position 3 or 4,
R_2 is ceramide comprising a hydroxyl fatty acid or an analog of a ceramide comprising a hydroxyl fatty acid and
R_1 is Galα, Galβ, GalNAcβ, GlcNAcβ or longer oligosaccharide comprising one of these residues at the reducing end or Neu5Xα with the proviso that preferably when R_1 is GlcNAcβ or Galα or Neu5Xα then x is 3 and Neu5X is sialic acid preferably Neu5Ac or Neu5Gc.

The present invention is directed to substances and compositions comprising polyvalent conjugates of lactose receptor according to the invention and especially polyvalent conjugates of a mimetic structure of lactosylceramide according to the present invention. Especially polyvalent conjugates of mimetic structures of lactosylceramide are preferred when the lactosylceramide or mimetic structure of lactosylceramide is linked to a polysaccharide, optionally through a spacer group. In a specific embodiment the use of polyvalent conjugates are preferred over the use of lactosylceramide glycolipids. Use of glycolipids is more difficult as there is need to prevent the diffusion of the receptors to tissues. The prevention can be, however, achieved for example by incorporating the glycolipids in medical carbon matrix or in a stabile membrane or micellar structures.

It is realized that two or even three or more receptor binding specificities according to the invention can be presented by a single lactosylceramide receptor.

The present invention is also directed to the use of lactosylceramide comprising hydroxylfatty acids and analogs and derivatives thereof for therapy of gastrointestinal diseases, especially diarrheas and hepatobiliary diseases and more specifically diseases caused by *zHelicobacter* bacteria. In a preferred embodiment the present invention is directed to the use of a milk fraction comprising lactosylceramide comprising a hydroxylfatty acid. The milk is preferentially from a dairy animal such as a cow or any other dairy animal or milk producing animal which produces hydroxyl fatty acid-containing lactosylceramide. The prior art discussed above has been directed to the use of some milk glycolipids but the prior art does not realize the usefulness of the hydroxylfatty acid-containing glycolipids against diarrhea-causing *zHelicobacter* bacteria. The lactosylceramide receptors according to the present invention are especially useful for functional food or feeds as nutritional additives.

Lacto-receptors

Preferred lacto series receptors comprise one or several oligosaccharide sequences according to the Formula

$$Gal\beta3GlcNAc[\beta3Gal\{\beta4Glc(NAc)_{n1}\}_{n2}]_{n3} \qquad (VII)$$

wherein n1, n2, and n3 are independently integers 0 or 1. In preferred embodiments at least n3 is 1. Most preferred oligosaccharide sequences referred here as high affinity receptors include oligosaccharide sequences Galβ3GlcNAcβ3Gal, Galβ3GlcNAcβ3Galβ4Glc, Galβ3GlcNAcβ3Galβ4GlcNAc and Galβ3GlcNAcβ3Galβ3GlcNAc. The use of lactotetraose Galβ3GlcNAcβ3Galβ4Glc, optionally with other milk oligosaccharide such as Galβ4GlcNAcβ3Galβ4Glc and/or and/or GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc, is especially preferred for therapeutical uses and especially for food, feed, and other nutritional uses.

Neolacto-receptors

Preferred neolacto series receptors comprise one or several oligosaccharide sequences according to the Formula

$[GlcNAc\beta3]_{n1}Gal\beta4GlcNAc[\beta3Gal\{\beta4Glc(NAc)_{n2}\}_{n3}]_{n4}$ (VIII)

wherein n1, n2, n3 and n4 are independently integers 0 or 1, when n1 is 1, the non-reducing terminal GlcNAc according to the formula can be further substituted by another monosaccharide residue or oligosaccharide residues, preferably by Galβ4 or GlcNAcβ3Galβ4. In preferred embodiments of the invention at least n4 is 1 or n1 is 1. Most preferred oligosaccharide sequences referred here as high affinity receptors include oligosaccharide sequences GlcNAcβ3Galβ4GlcNAc, Galβ4GlcNAcβ3Gal, Galβ4GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4GlcNAc, GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc, and GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAc. Preferred GlcNAcβ3Galβ4GlcNAc–structures include oligosaccharide sequences, which are β6-linked from the reducing end, especially GlcNAcβ3Galβ4GlcNAcβ6Gal, GlcNAcβ3Galβ4GlcNAcβ6GalNAc, GlcNAcβ3Galβ4GlcNAcβ6GlcNAc, GlcNAcβ3Galβ4GlcNAcβ6Glc and GlcNAcβ3Galβ4GlcNAcβ6Man. The use of neolactotetraose Galβ4GlcNAcβ3Galβ4Glc is especially preferred for therapeutic uses and especially for food, feed, and other nutritional uses.

A preferred embodiment of the invention is directed to uses of neolacto binding sequences comprising terminal-GlcNAc structures such as GlcNAcβ3Galβ4GlcNAc and GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc. It is realized that even the terminal disaccharide sequence GlcNAcβ3Gal can be used according to the invention, though with less activity. It is also found for the first time that linear β3-linked poly-N-acetyllactoamines, Galβ4GlcNAc[β3Galβ4GlcNAc]$_n\beta$3Galβ4Glc where in n is integer and n>=1, are receptors for *zHelicobacter* strains, the terminal Gal can be substituted by other monosaccharide residues, for example Neu5Xα3 or GlcNAcβ3. Preferred monovalent inhibitors comprises GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc, which has been reported from milk of buffalo, the common milk oligosaccharide Galβ4GlcNAcβ3Galβ4Glc and mixtures comprising GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc and Galβ4GlcNAcβ3Galβ4Glc.

Ganglio-receptors

Preferred ganglioseries receptor comprises oligosaccharide sequences according to the Formula

$$[Gal\beta3]_{n1}GalNAc[\beta4Gal\{\beta4Glc\}_{n2}]_{n3} \quad (IX)$$

wherein n1, n2 and n3 are independently integers 0 or 1, preferably with the proviso that at least n1 or n3 is 1 and with the proviso that no sialic acids are linked to the oligosaccharide sequence.

More preferably the ganglio receptors are according to the formula

$$[Gal\beta3]_{n1}GalNAc\beta4Gal\beta4\{Glc\}_{n2} \quad (X)$$

wherein n1, and n2 are independently integers 0 or 1, preferably with the proviso that at least n2 or n3 is 1.

The preferred oligosaccharide sequences are Galβ3GalNAcβ4Galβ4Glc, Galβ3GalNAcβ4Gal, Galβ3GalNAc, GalNAcβ4Gal and GalNAcβ4Galβ4Glc and even more preferred sequences includes Galβ3GalNAcβ4Galβ4Glc, GalNAcβ4Gal and GalNAcβ4Galβ4Glc.

The screening of wide variety of ganglioseries and comparison of the structures in examples of the present invention allows the determination of Galβ3GalNAc as a novel preferred novel receptor oligosaccharide sequences of the ganglioseries receptor oligosaccharide sequences. The data indicates that even terminal Galβ3GalNAc in GM1-sequence can bind to *zHelicobacter*. The binding to the terminal disaccharide has previously not been demonstrated and the tetrasaccharide epitopes may be used in formulations which allows more effective presentation of the terminal disaccharide. According to one embodiment of the invention, the Galβ3GalNAc is preferably not β4 linked to lactose. The disaccharide epitope is in general cheaper to produce than the tetrasaccharide epitope. More preferably the oligosaccharide sequence is Galβ3GalNAcβ with the proviso that the disaccharide epitope is not linked to lactose or Galβ3GalNAcβ4Gal, with the proviso that the reducing end Gal is not linked to

glucose. Another cost effective oligosaccharide sequence is GalNAcβ4Gal which is also cheaper to produce than the tetrasacccharide. Similarily the trisaccharide GalNAcβ4Galβ4Glc can be effectively produced from lactose for example by enzymatic methods.

Sialic acid receptor

In the broadest sense the sialic acid receptor may be any sialic acid in natural type glycoconjugates. The sialic acid is preferably N-acetyl-neuraminic acid. In another embodiment the sialic acid is N-glycolyl-neuraminic acid.

The present invention recognizes a specific sialic acid which can bind effectively to the pathogens, especially *zHelicobacter* bacteria.

The preferred sialic acid receptor oligosaccharide sequences are according to the Formula

SAαpGalβ (XI)

And more preferably according to formula

SaαpGalβ4Glc(NAc)$_n$ (XII)

SA is sialic acid preferably N-acetylneuraminic acid, in another embodiment, SA is Neu5X wherein independently X is either Ac or Gc meaning that the sialic acic is either Neu5Ac or Neu5Gc,

n is 0 or 1.

Preferred oligosaccharide sequences include one or several of the group: Neu5Xα3Galβ3(Fucα4)GlcNAc, and Neu5Xα3Galβ4(Fucα3)GlcNAc, Neu5Xα3Galβ4(Fucα3)Glc, Neu5Xα3Galβ3GlcNAc, Neu5Xα3Galβ4GlcNAc, Neu5Xα3Galβ4Glc, and Neu5Xα6Galβ4GlcNAc, Neu5Xα6Galβ4Glc wherein X is either Ac or Gc. The use of one or several of the milk type oligosaccharides such as Neu5Xα3Galβ3GlcNAcβ3Galβ4Glc, Neu5Xα3Galβ4GlcNAcβ3Galβ4Glc, sialyl-

Lewis a hexasaccharide Neu5Xα3Galβ3(Fucα4)GlcNAcβ3Galβ4Glc or sialyl-Lewis x hexasaccharide Neu5Xα3Galβ4(Fucα3)GlcNAcβ3Galβ4Glc or sialyl-lactoses Neu5Xα3Galβ4(Fucα3)Glc, Neu5Xα3Galβ4Glc Neu5Xα6Galβ4Glc is especially preferred for therapeutical uses and especially for food, feed, and other nutritional uses. Most preferred sialic acid receptors comprise oligosaccharide sequences selected from the group NeuNAcα3Gal, NeuNAcα6Gal, NeuNAcα3Galβ4GlcNAc and NeuNAcα6Galβ4GlcNAc. Most preferred milk oligosaccharides includes the α3sialylated structures NeuNAcα3Galβ4GlcNAc and Neu5Acα3Galβ4GlcNAcβ3Galβ4Glc and mixtures thereof and as a separate embodiment α6sialylated structures NeuNAcα6Galβ4GlcNAc and Neu5Acα6Galβ4GlcNAcβ3Galβ4Glc and mixtures thereof.

When the oligosaccharide sequences are used in human applications, it is preferred in a specific embodiment of the invention to use a natural human type of oligosaccharides wherein X is Ac and Neu5X is therefore Neu5Ac

The present invention is also directed to polysialic acid-type oligosaccharide substances or polysialic acid compositions for therapeutic uses or for use as medicine. The substances and compositions are especially directed for non-vaccine therapeutic uses and medicines. The present invention is also directed to the use of polysialic acid-type oligosaccharide substances for the preparation of medicines and therapeutic compositions against diarrheas and compositions for *ex vivo* uses as described by the present invention.

Use of partial oligosaccharide sequences

In a separate embodiment one or several of the oligosaccharide sequences according to the present invention is/are replaced by a partial oligosaccharide sequences. The partial oligosaccharide sequence is in general less effective but can be used in higher concentrations. The partial oligosaccharide sequences are preferentially monosaccharides and more preferentially non-reducing pyranose formed monosaccharide residues having the same anomeric sructure as a terminal monosaccharide residue in a oligosaccharide sequence according to the present invention, more preferably the non-reducing pyranose formed monosaccharide residue is linked to a polyhydroxyl substance partially mimicking next monosaccharide of the corresponding oligosaccharide

sequence. In a preferred embodiment the polyhydroxyl susbtance is a non-carbohydrate substance and most preferably the polyhydroxyl substance is a flexible hydrophilic linker described by Formula 2 in this invention. Preferred partial oligosaccharide sequences include polyvalent conjugates and soluble polyvalent conjugates of the partial oligosaccharide sequences as described for the other receptor oligosaccharide sequences.

The partial oligosaccharide sequence is preferentially Manα, and more preferentially non-reducing pyranose formed Manα linked to a polyhydroxyl substance partially mimicking next monosaccharide of the corresponding oligosaccharide sequence. In another embodiment the partial oligosaccharide sequences are chosen from the group Galβ, Galα, GlcNAcβ and GalNAcβ optionally linked to a polyhydroxyl substance partially mimicking next monosaccharide of the corresponding oligosaccharide sequence. The partial oligosacharide sequences are preferably used together with low cost oligosaccharide sequences. Preferably one partial oligosaccharide sequence in pyranose form is used together with at least one, and preferably with two oligosaccharide sequences, and most preferably with three oligosaccharide sequences, according to the present invention. In another embodiment at least two partial oligosaccharide sequences are used with at least one oligosaccharide sequence according to the present invention. The partial oligosaccharide sequences are preferred for therapeutic uses according to the present invention, especially for feed and food uses.

Defining most relevant carbohydrate binding specificities with regard to the natural infection cascade

As described below, any carbohydrate specificity or specificities present on a pathogen cell surface can be used to inhibit the binding of a pathogen, for example by soluble polyvalent carbohydrates using the covering method as described by the present invention.

However, it is especially preferred to target such carbohydrate binding specificities which are directed to relevant receptors on the tissue which is infected. This is a preferred method when monovalent substances according to the invention are used. When soluble polyvalent conjugates are used for inhibition of a pathogen cell, and the most relevant carbohydrate specificities are used, the polyvalent or even oligovalent

conjugate need not be large like the conjugates which are used for achieving the sterical inhibition of other receptor interactions according to the invention. The present invention demonstrates several novel carbohydrate receptor structures on glycoproteins of human intestine and connects these to the binding specificities shown by assays. In some cases the binding specificity of a certain intestinally pathogenic *zHelicobacter* has been described but only the present invention shows its relevance to the infection by characterizing the natural receptor saccharides in human intestine. In a few cases combination of receptor structures and possible binding have been separately indicated to a certain extent. However, in these cases the characterization of potential receptors and binding specificities allow design of more effective receptor oligosaccharide sequences.

Most relevant carbohydrate binding specificities of human intestine

Analysis of glycoproteins from human intestine revealed unexpectedly several interesting carbohydrate receptor structures. Combination of bacterial binding data and the presence of receptor allows defining of the biologically most useful therapeutic and diagnostic structures. The binding specificities under this category also aim to use receptor specificities, which are not so common in the normal useful bacterial flora.

Sialic acid comprising receptors and sialic acid binding specificities

Potential sialic acid comprising structures have not been characterized from human intestinal glycoproteins. The present invention shows sialylated structures and binding of diarrhea-causing *zHelicobacter* to these structures. The sialic acid binding specificity of any diarrhea-causing *zHelicobacter* has not been characterized in detail. The minor reports with only a few strains do not reveal the major sialic acid binding specificities according to the present invention and these specificities have not been connected with the receptor structures.

Lacto-receptors and Neolacto-receptors

Present invention was able to demonstrate the presence of protein linked lacto- and neolacto-type first contact receptors in human gastrointestinal tract. The data show that the lacto-receptors and neolacto-receptors are present and available for pathogen

binding, showing the relevance of the receptors for pathogenesis, especially with regard to *zHelicobacter* infections.

General binding specificities also commonly present in normal flora

Lactosylceramide and ganglio-receptors are known to bind normal bacterial flora. The use of these receptors may also reduce normal flora or probiotic bacteria and are therefor more preferred to be used in combination with probiotic bacteria or probiotic substances.

These receptors belong to the second contact receptor category and are most useful in connection to the other receptors described to be in the first contact receptors when the most effective treatment is needed. Galα4Gal structures can be also considered partially as normal flora binding structures. In a separate embodiment Galα4Gal structures are used together with probiotic bacteria.

The lactosylceramide binding

The glycolipid receptor lactosylceramide comprising hydroxyl fatty acids is a novel receptor activity for *zHelicobacter*. This specificity includes 3'modified lactosylceramides, structures having modification or the elongation of the oligosaccharide chain on carbon 3 of the Gal residue in lactosylceramide. Lactosylceramide comprising hydroxyl fatty acids is known from intestinal tissue and considered as a general receptor for *zHelicobacter*.

Inhibition of pathogens by monovalent receptors

It is generally believed that the carbohydrate bindings to their receptors and especially the bindings of pathogenic bacteria are quite weak as monovalent interactions. It has been shown that for example binding of the Shiga-like toxin of *E. coli* to cultivated cells, can be only inhibited by very high density polyvalent carbohydrate conjugates of the Galα4Gal-sequence.

An approach using monovalent oligosaccharide sequences could save costs of synthesis when the construct is prepared. Polyvalent conjugates may also comprise non-natural and non-biodegradable linker structures which may cause side effects or regulatory problems. In general it is desired that the monovalent oligosaccharide should be

active at low concentrations that would allow cost effective use of the oligosaccharide. The monovalent oligosaccharide means here also monovalent conjugates of the oligosaccharide, for example glycosylamines or glycosylamides or methyl glycosides or other glycosides including other N-glycosides, C-glycosides or S-glycosides, or for example active derivatives in which the reducing end is modified by reduction or reductive amination. If the reducing –end monosaccharide residue is reduced, it may be used as a spacer outside of the binding active carbohydrate epitope. Such an approach would require the use of an oligosaccharide which is at least one monosaccharide residue longer than the desired receptor epitope in the oligosaccharide sequence.

The present invention demonstrates that unexpectedly high affinity monovalent binding activities can be found and that monovalent carbohydrates can be used in relatively low concentrations to inhibit the bindings. Preferred monovalent substances comprise one or several terminal non-reducing end sequences chosen from the group: alpha-linked sialic acid, Neu5Acα, Neu5Acα3, Neu5Acα6, Neu5Acα3Gal, Neu5Acα6Gal, Neu5Acα9Neu5Ac, Neu5Acα8Neu5Ac, Galβ3GalNAc, GalNAcβ4Gal, Galβ3GlcNAc, Galβ4GlcNAc, GlcNAcβ3Gal, and GlcNAcβ3Galβ4GlcNAc.

More preferentially the monovalent substance or substances comprise(s) one or several terminal non-reducing end sequences chosen from the group: Neu5Acα3Gal, Neu5Acα6Gal, Neu5Acα3Galβ4Glc, Neu5Acα6Galβ4Glc, Neu5Acα8Neu5Acα8Neu5Ac, Neu5Acα8Neu5Ac, Neu5Acα8/9Neu5Acα8/9Neu5Ac, Galβ3GalNAcβ4Galβ4Glc, GalNAcβ4Galβ4Glc, Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, GlcNAcβ3Galβ4GlcNAc, Neu5Xα3Galβ3GlcNAcβ3Galβ4Glc, Neu5Xα3Galβ4GlcNAcβ3Galβ4Glc, Neu5Xα3Galβ4Glc Neu5Xα6Galβ4Glc.

Most preferentially the monovalent substance one or several terminal non-reducing end sequences chosen from the group: Neu5Acα3Galβ3GlcNAcβ3Galβ4Glc, Neu5Acα3Galβ4GlcNAcβ3Galβ4Glc, Neu5Acα3Galβ3(Fucα4)GlcNAcβ3Galβ4Glc, Neu5Acα3Galβ4Glc, Neu5Acα6Galβ4Glc, Galβ3GalNAcβ4Galβ4Glc, Gal-

NAcβ4Galβ4Glc, Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, and GlcNAcβ3Galβ4GlcNAc3Galβ4Glc.

This group comprises natural type asialo ganglioside sequences and oligosaccharides which are present in animal or human milk.

Synergistic effects of manipulating several carbohydrate receptor bindings

The first synergistic effect is the unexpectedly high efficiency in inhibition or binding to a single pathogen which represent several adhesins binding to cell surfaces of a patient. In traditional inhibition attempts with single oligosaccharide epitopes the pathogen usually has additional carbohydrate binding specificities which may allow it to survive in the tissue. The prevention or inhibition of the binding is more effective when as many binding components as possible are inhibited. When a polyvalent conjugate is used, the highest affinity part of the conjugate targets possible receptor oligosaccharide sequences with lower affinity to the surface of the pathogen. When the inhibition covers most of the binding mechanisms of the pathogen, the inhibition exceeds a threshold value allowing the pathogen mass to be flushed away by liquids of the tissue, causing a dramatic preventive effect against the pathogen. When the invention is used to inhibit simultaneously a microbe and a toxin involved in the same disease, the disease is relieved by two means, *i.e.* removal of both the bacterium and the toxin.

In this invention the terms "analog" and "derivative" are defined as follows. According to the present invention it is possible to design structural analogs or derivatives of the *zHelicobacter* binding oligosaccharide sequences. Thus, the invention is also directed to the structural analogs of the substances according to the invention. The structural analogs according to the invention comprise the structural elements important for the binding of *zHelicobacter* to the oligosaccharide sequences. For design of effective structural analogs it is important to know the structural element important for the binding between *zHelicobacter* and the saccharides. The important structural elements are preferably not modified or these are modified by very close mimetics of the important structural element. These elements preferably include the 4-, and 6-hydroxyl groups of the Galβ4 residue in the trisaccharide and oligosaccharide epitopes. Also the positioning of the linkages between the ring structures is an important structural element. For a high affinity binding the acetamido group or acetamido mimicking group is preferred in the position corresponding to the acetamido group of the reducing end-

GlcNAc of the di- or trisaccharide epitopes. Acetamido group mimicking group may be another amide, such as alkylamido, arylamido, secondary amine, preferentially N-ethyl or N-methyl, O-acetyl, or O-alkyl for example O-ethyl or O-methyl.

The structural derivatives according to the invention are oligosaccharide sequences according to the invention modified chemically so that the binding to the *zHelicobacter* is retained or increased. According to the invention it is preferred to derivatize one or several of the hydroxyl or acetamido groups of the oligosaccharide sequences. The invention used to describe several positions of the molecules which could be changed when preparing the analogs or the derivatives. Preferred derivatives of the receptor oligosaccharide sequences according to the present invention include reducing-end derivatives of the oligosaccharide sequences. Multiple derivatization methods are known to link oligosaccharides to other carbohydrates, aglycon molecules or various carriers. The C1-carbon of the reducing end monosaccharide residue can be linked through a sulphur, carbon or nitrogen atoms to other carbohydrates, aglycon molecules or various carriers, especially polyvalent carriers. Methods such as reductive amination can be used when the pathogen binding carbohydrate epitope is not destroyed by opening the reducing end monosaccharide residue. Derivatives of acetamido groups are also preferred. Acetamido- groups can be deacetylated and derivatized for example by other carboxylic acids, the acetamido-derivatives can be screened for better pathogen binding. The derivatives can also be produced from precursors of the oligosaccharide to be derivatized for example from oligosaccharide sequences comprising hexosamine-residues. Methods to produce oligosaccharide analogs for the binding of a lectin are well known. For example, numerous analogs of sialyl-Lewis x oligosaccharide have been produced, representing the active functional groups on different scaffolds (see page 12090, Sears and Wong 1996). Similarly, analogs of heparin oligosaccharides has been produced by Sanofi corporation and sialic acid-mimicking inhibitors, such as Zanamivir and Tamiflu (Relenza), for the sialidase enzyme by numerous groups. Preferably the oligosaccharide analog is built on a molecule comprising at least one six- or five-membered ring structure, more preferably the analog contains at least two ring structures comprising 6 or 5 atoms.

In mimicking structures monosaccharide rings may be replaced rings such as cyclohexane or cyclopentane, aromatic rings including benzene ring, heterocyclic ring structures may comprise besides oxygen for example nitrogen and sulphur atoms. To lock the active ring conformations the ring structures may be interconnected by tolerated linker groups. Typical mimetic structures may also comprise peptide analog-structures for the oligosaccharide sequence or part of it.

The effects of the active groups to binding activity are cumulative and lack of one group could be compensated by adding an active residue on the other side of the molecule. Molecular modelling, preferably by a computer can be used to produce analog structures for the *zHelicobacter* binding oligosaccharide sequences according to the invention. The results from the molecular modelling of several oligosaccharide sequences are given in examples and the same or similar methods, besides NMR and X-ray crystallographic methods, can be used to obtain structures for other oligosaccharide sequences according to the invention. It is also noted that the monovalent, oligovalent or polyvalent oligosaccharides can be activated to have higher activity towards the lectins by making derivatives of the oligosaccharide by combinatorial chemistry. When the library is created by substituting one or a few residues in the oligosacharide sequence, it can be considered as a derivative library, alternatively when the library is created from the analogs of the oligosaccharide sequences described by the invention. A combinatorial chemistry library can be built on the oligosaccharide or its precursor or on glycoconjugates according to the invention. For example, oligosaccharides with variable reducing ends can be produced by so called carbohydrid technology. In a preferred embodiment a combinatorial chemistry library is conjugated to the *zHelicobacter* binding substances described by the invention. In a more preferred embodiment the library comprises at least 6 different molecules. Such library is preferred for use of assaying microbial binding to the oligosaccharide sequences according to the invention. Amino acids or collections of organic amides are commercially available and can be used for the synthesis of combinatorial library of acetamido analogs. A high affinity binder could be identified from the combinatorial library for example by using an inhibition assay, in which the library compounds are used to inhibit the bacterial binding to the glycolipids or glycoconjugates described by the invention. Structural analogs and derivatives preferred according to the invention can inhibit the binding of the *zHelicobacter* binding oligosaccharide sequences according to the invention to *zHelicobacter*.

In the present invention the binding epitope, receptor or pathogen receptor or pathogen inhibitor by other words, especially for diarrheagenic *zHelicobacter*, are described as oligosaccharide sequences. The oligosaccharide sequence defined here can be a part of

a natural or synthetic glycoconjugate or a free oligosaccharide or a part of a free oligosaccharide. Such oligosaccharide sequences can be bonded to various monosaccharides or oligosaccharides or polysaccharides on polysaccharide chains, for example, if the saccharide sequence is expressed as part of a bacterial polysaccharide. Moreover, numerous natural modifications of monosaccharides are known as exemplified by O-acetyl or sulphated derivative of oligosaccharide sequences. The *zHelicobacter* receptor oligosaccharide sequence defined here can comprise the oligosaccharide sequence described as a part of a natural or synthetic glycoconjugate or a corresponding free oligosaccharide or a part of a free oligosaccharide. The *zHelicobacter* receptor oligosaccharide sequence can also comprise a mix of the *zHelicobacter* receptor oligosaccharide sequences. In a preferred embodiment the the oligosaccharide sequences according to the present invention are non-reducing terminal oligosaccharide sequences, which means here that the oligosaccharide sequences are not linked to other monosaccharide or oligosaccharide structures except optionally from the reducing end of the oligosaccharide sequence. The oligosaccharide sequence when present as conjugate is preferably conjugated from the reducing end of the oligosaccharide sequence, though other linkage positions which are tolerated by the pathogen binding can also be used. In a more specific embodiment the oligosaccharide sequence according to the present invention means the corresponding oligosaccharide residue which is not linked by natural glycosidic linkages to other monosaccharide or oligosaccharide structures. The oligosaccharide residue is preferably a free oligosaccharide or a conjugate or derivative from the reducing end of the oligosaccharide residue.

The pathogen receptor oligosaccharide sequences can be synthesized enzymatically by glycosyltransferases, or by transglycosylation catalyzed by glycosidase or transglycosidase enzymes (Ernst *et al.*, 2000). Specifities of these enzymes and the use of cofactors can be engineered. Specific modified enzymes can be used to obtain more effective synthesis, for example, glycosynthase is modified to do transglycosylation only. Organic synthesis of the saccharides and the conjugates described herein or compounds similar to these are known (Ernst *et al.*, 2000). Saccharide materials can be isolated from natural sources and modified chemically or enzymatically into the pathogen receptor compounds. Natural oligosaccharides can be isolated from milks produced by various ruminants. Transgenic organisms, such as cows or microbes, expressing glycosylating enzymes can be used for the production of saccharides.

The pathogen receptor substances, preferably in oligovalent or clustered form, can be used to treat a disease or condition caused by the presence of the pathogen, preferably diarrhea causing *zHelicobacter*. This is done by using the *zHelicobacter* receptor sub-

stances for anti-adhesion, i.e. to inhibit the binding of *zHelicobacter* to the receptor epitopes of the target cells or tissues. When the *zHelicobacter* binding substance or pharmaceutical composition is administered it will compete with receptor glycoconjugates on the target cells for the binding of the bacteria. Some or all of the bacteria will then be bound to the *zHelicobacter* receptor substance instead of the receptor on the target cells or tissues. The bacteria bound to the *zHelicobacter* receptor substances are then removed from the patient (for example by the fluid flow in the gastrointestinal tract), resulting in reduced effects of the bacteria on the health of the patient. Preferably the substance used is a soluble composition comprising the *zHelicobacter* receptor substances. The substance can be attached to a carrier substance which is preferably not a protein. When using a carrier molecule several molecules of the *zHelicobacter* receptor substance can be attached to one carrier and inhibitory efficiency is improved.

According to the invention it is possible to incorporate the *zHelicobacter* receptor substance, optionally with a carrier, in a pharmaceutical composition, which is suitable for the treatment of a condition due to the presence of *zHelicobacter* in a patient or to use the *zHelicobacter* binding substance in a method for treatment of such conditions. Examples of conditions treatable according to the invention are and related gastrointestinal diseases, all, at least partially, caused by the *zHelicobacter* infection.

The pharmaceutical composition containing the pathogen receptor preferably diarrheagenic *zHelicobacter*-receptor substance may also comprise other substances, such as an inert vehicle, or pharmaceutically acceptable carriers, preservatives *etc*, which are well known to persons skilled in the art. The pathogen receptor, preferably diarrheagenic *zHelicobacter*-receptor-substance, can be administered together with other drugs such as antibiotics used against the pathogen or specifically *zHelicobacter*.

The pathogen receptor, preferably diarrheagenic *zHelicobacter*-receptor substance or pharmaceutical composition containing such substance, may be administered in any suitable way, although an oral administration is preferred.

The receptor oligosaccharide sequences according to the present invention are aimed for use in inhibition against pathogens, especially pathogenic bacteria, and the receptor oligosaccharide sequences are also referred as pathogen inhibiting oligosaccharide sequences. In more specific embodiments the pathogen is diarrhea causing zHelicobacter and the receptor oligosaccharides are also referred as pathogen inhibiting oligosaccharide sequences or as *zHelicobacter* receptor substances. The naming of the spe-

cific receptor oligosaccharide sequences and other longer terms may vary with regard to use of dash or capital letter as first letter, for example "lacto-receptor" and "lacto receptor" and "Lacto-receptor" and "Lacto receptor" mean the same.

The term "purified fraction" used herein relates to purified or isolated oligosaccharide fraction from natural or synthetic sources. In a preferred embodiment the amount of the active oligosaccharide sequence or oligosaccharide sequences is analysed and/or controlled from the fraction, optionally the amounts of other related carbohydrate structures are also analysed. The purified fraction has reduced amount of inactive substances originating from the source of the fraction, for example protein, monosaccharide precursors, lactose, or fat. Potentially harmful substances, such as harmful chemicals from synthesis, allergenic proteins, or substances considered ethically harmful, for example by religious or diet culture reasons, are removed to a level where these are not harmful in the final product. For medical use the purified fraction is preferably essentially pure (i.e. a purity of 98 % or better), or non-relevant substances are controlled and comprise preferably at least less than half of the mass of the purified fraction, more preferably less than 20% of the mass of the purified fraction and most preferably less than 5 % of the mass of the purified fraction. In a preferred embodiment of the invention, the production of the purified fraction from animal milk or milks involves at least partial removal of milk protein and/or fat. The purification may comprise filtration methods, such as gel filtration or ultrafiltration, as well as drying and/or concentrating steps. For non-medical use, the purified fraction is preferably essentially pure or the non-relevant substances comprise preferably at least less than 95 % of the mass of the purified fraction, more preferably less than 75% of the mass of the purified fraction and most preferably less than 25 % of the mass of the purified fraction. The purified fraction may be used as such or together with other ingredients of the desired product.

The term "treatment" used herein relates both to treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The treatment may be either performed in a acute or in a chronic way.

The term "patient", as used herein, relates to any human or a cattle or household pet mammal in need of treatment according to the invention. The present infection is especially directed for the treatment of intestinal infections, especially diarrheas, when the patient is a human patient. Preferred pet animals includes cats, dogs and rodents, most preferably the pet is a cat or dog.

It is also possible to use the pathogen receptor preferably diarrheagenic *zHelicobacter*-receptor substance in screening for substances that bind to the receptor substance, for example for screening of carbohydrates (by carbohydrate-carbohydrate interactions) that bind to the *zHelicobacter* receptor substance. The screening can be done for example by affinity chromatography.

Furthermore, it is possible to use substances specifically binding or inactivating the *zHelicobacter* receptor substances present on human tissues and thus prevent the binding of *zHelicobacter*. (Examples of such substances include plant lectins such as *Erythrina cristagalli* and *Erythrina corallodendron* (Teneberg *et al.*, 1994)??). When used in humans, the binding substance should be suitable for such use such as a humanized antibody or a recombinant glycosidase of human origin which is non-immunogenic and capable of cleaving the terminal monosaccharide residue/residues from the *zHelicobacter* receptor substances. However, in the gastrointestinal tract, many naturally occuring lectins and glycosidases originating for example from food are tolerated.

Nutritional, food and feed uses

Furthermore, it is possible to use the pathogen receptor oligosaccharide sequences or *zHelicobacter* receptor oligosaccharide as part of a nutritional composition including food- and feedstuff. It is preferred to use the receptor oligosaccharide sequences according to the present invention in single substances or as single substances and more preferably in a composition comprising at least two receptor oligosaccharide sequences from different groups according to the present invention for nutritional compositions, foods or feed stuffs. It is preferred to use the *zHelicobacter* receptor oligosaccharide sequences as substances or compositions as a part of so called functional or functionalized food. The said functional food has a positive effect on the person's or animal's health by inhibiting or preventing the binding of *zHelicobacter* to target cells or tissues. The *zHelicobacter* receptor substance or composition can be a part of a defined food or functional food composition. The functional food can contain other acceptable food ingredients accepted by authorities such as Food and Drug Administration in the USA. The *zHelicobacter* receptor substance or composition can also be

used as a nutritional additive, preferably as a food or a beverage additive to produce a functional food or a functional beverage. The food or food additive can also be produced by having, *e.g.*, a domestic animal such as a cow or other animal produce the *zHelicobacter* receptor substance or composition in larger amounts naturally in its milk. This can be accomplished by having the animal overexpress suitable glycosyltransferases in its milk. A specific strain or species of a domestic animal can be chosen and bred for larger production of the *zHelicobacter* receptor substance or composition. The *zHelicobacter* receptor substance or composition for a nutritional composition or nutritional additive can also be produced by a micro-organism such as a bacteria or a yeast.

Present invention is especially directed to use of the substances in animal feed including feeds of cats and dogs in risk of infection by *zHelicobacter*.

It is especially useful to have the *zHelicobacter* receptor substance or composition as part of a food for an infant, preferably as a part of an infant formula. Many infants are fed by special formulas in replacement of natural human milk. The formulas may lack the special lactose based oligosaccharides of human milk, especially the elongated ones such as lacto-N-neotetraose, Galβ4GlcNAcβ3Galβ4Glc, lacto-N-tetraose, Galβ3GlcNAcβ3Galβ4Glc, and derivatives thereof. The lacto-N-tetraose, lacto-N-neotetraose para-lacto-N-hexaose (Galβ3GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc and para-lacto-N-neohexaose (Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc) as well as Galβ3Galβ4Glc are known from human milk and can therefore be considered as safe additives or ingredients in an infant food. Sialylated and/or fucosylated human milk oligosaccharides and buffalo milk oligosaccharide GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc, described as pathogen receptors according to the present invention, are also preferred for functional foods and infant formulas. It is preferred to use combinations comprising at least two of the milk oligosaccharides. Diarrhea causing *zHelicobacter* is especially infective with regard to infants or young children, and considering the diseases it may later cause it is reasonable to prevent the infection.

Preferred concentrations for human milk oligosaccharides in functional food to be consumed (for example, in reconstituted infant formula) are similar to those present in natural human milk. It is noted that natural human milk contains numerous free oligosaccharides and glycoconjugates (which may be polyvalent) comprising the oligosaccharide sequence(s) described by the invention, wherefore it is possible to use even higher than natural concentrations of single molecules to get stronger inhibitory effect

against *zHelicobacter* without harmful side effects. Natural human milk contains lacto-N-neotetraose at least in range about 10 – 210 mg/l with individual variations (Nakhla *et al.*, 1999). Consequently, lacto-N-neotetraose is preferably used in functional food in concentration range 0,01 – 10 g/l, more preferably 0,01 – 5 g/l, most preferably 0,1 – 1 g/l. Approximately 2-5 times higher amounts of lacto-N-tetraose can be used. Alternatively, the total concentration of the saccharides used in functional food is the same or similar to the total concentration of natural human milk saccharides, which bind *zHelicobacter* like the substances or composition described, or which contain the binding epitope/oligosaccharide sequence indicated in the invention.

Infant formulas also comprise, beside substances or compositions according to the present invention, other substances used in infant formulas such as fractions from ruminant milks such as proteins from whey or soy protein preparations or protein hydrolysates. The infant formula may also comprise other carbohydrates useful or accepted for infant formulas such as lactose or galactose oligosaccharides.

Diagnostic and analytical uses related to therapeutical uses

Furthermore, it is possible to use the *zHelicobacter* binding oligosaccharide receptors according to the present invention in the diagnosis of a condition caused by an *zHelicobacter* infection. Diagnostic uses also include the use of the *zHelicobacter* binding substance for typing of *zHelicobacter*. The typing of *zHelicobacter* with regard to binding of the carbohydrate receptors according to the present invention can be used to determine effective combination of therapeutic carbohydrates for a specific diarrheagenic *zHelicobacter* strain. This can be useful for making specific lower cost theraphies for local infections, the profiles of carbohydrate bindings of major diarrhea causing *zHelicobacter* may differ in different geographic locations and during epidemies.

It is also realized that substances according to the invention can be used as anti-infectives to block zHelicobacter binding and to prevent infections *ex vivo*, examples include food preservatives, mouth hygiene products, topical, washing or cosmetic products comprising a substance as defined in any of the claims 1-13.

The preferred indications according to the present invention

The present invention is directed to various therapeutic, preventive and diagnostic uses of the oligosaccharide sequences according to the invention. The present inven-

tion is especially directed to the treatment in the presence of the following pathogens and to the prevention of the following diseases.

Zoonotic Helicobacter species

The present invention is specifically directed to Helicobacter species causing gastric infections to human and animal living in close contact with human. The zoonotic species also cause other diseases as described by the invention The species of bacteria have varying zoonotic potential. The bacteria from animals living ind close contact with human includes the large group of enterohepatic Helicobacters from H. pullorum to H. westmaedii and gastric species from H. suis to H.salomonis, preferably also including bovine species (H. bovis) and monkey species fig. 1 Dewhurst et al. 2000. The species of bacteria form homologous groups known to zoonotically infect human.This grouping does not include H. mustelae type "wild animal" species, less interesting as theraphy targets. These H.elicobacters form homologous groups known to containg zoonotic activities. Moreover the present invention describes the carbohydrate binding activities allowing the bacteria to spread. The species are different from H. pylori having Lewis b and/ more pronounced sialic acid based infection mechanisms. The invention is preferably directed to inhibition to the Helicobacters known to cause zoonotic infections. The preferred species includes group of "gastrospirilla" bacteria zoonotic cat and dog pathogens H. felis- H. bizzezeronii- and H. salomonis, which are same or very similar to a group of human infecting bacteria named in human H. heilmannii and another type of H.heilmannii resembles closely candidatus H. suis, a pig Helicobacter. Yet another zoonotic group includes species characterized as Flexipira rappini isolated from aborted lambs, dog an and human faeces and pig intestine. H. bilis. Group of helicobacters called H. rappini has been also known to infect human, with similarity to H. bilis and H. trogantum. Especially zoonotic species includes also H. canis and H. pullorum (from poultry to human) (On 2001) and H. fenellilae, H. cinaedi, H. canadiens, H winghamensis and H westmaedi (Fox 2002).

Zoonotic enteric infections causing diarrhea and other enteric diseases

The present invention invention is directed to treatment and/or prevention of diarrheas caused by zoonotic *Helicobacter* species. In a preferred embodiment one or several of the carbohydrates are used for acute or preventive treatment of infections in animals

living in close contact with humans. The invention is specifically directed to treatments of pet animals infectable with zoonotically spreading *Helicobacter* species. Such infected pets have reported to infect human beings and cause diseases including diarrheas. In a specific embodiment the treatment is given to the human or animal that is suffering of weakened immune protection or immunodeficiency.

Zoonotic *Helicobacter* infections causing hepatobiliary disease

The present invention is directed to the treatment and/or prevention of hepatobiliary infection caused by zoonotic *Helicobacter* species. In a preferred embodiment one or several of the carbohydrates are used for acute or preventive treatments of infections in animals living in close contact with humans. The invention is specifically directed to the treatment of pet animals infectable with zoonotically spreading *Helicobacter* species. Such infected pets have been reported to infect human beings and cause diseases including hepatobiliary diseases. In a specific embodiment the treatment is given to the human or animal that is suffering of weakened immune protection or immunodeficiency.

Zoonotic Helicobacter infections causing gastric or hepatic disease

The present invention is directed to the treatment and/or prevention of gastric infections and diseases caused by zoonotic *Helicobacter* species. In a preferred embodiment one or several of the carbohydrates are used for acute or preventive treatments of infections in animals living in close contact with humans. The invention is specifically directed to the treatment of pet animals infectable with zoonotically spreading *Helicobacter* species. Such infected pets have been reported to infect human beings and cause diseases including gastric infections. In a specific embodiment the treatment is given to the human or animal that is suffering of weakened immune protection or immunodeficiency.

Enterohepatic Helicobacteria

The invention is primarily targeted to common binding specificity shared with enterohepatic *non-H.pylori Helicobacter* species. These species includes *H. canis*, *H. bilis* and *H. hepaticus*. The similar galactose based binding specificity profile towards human and animal glyconjugates is also observable with *H. fenelliae*, *H. rappini* and *H*

.pullorum. In general the ecologic niches in enterohepatic system allows an effective use of limited amount of receptor carbohydrates. The present invention identifies the major receptor carbohydrates useful for binding enterohepatic system of human and animals. In a specific embodiment the galactose binding specificity is further applicable for inhibition and binding assays with other enterohepatic *Helicobacter* species having the same infectivity profile in enterohepatic system of human and animals.

Zoonotic Helicobacteria causing gastric infection

The present invention is further directed to treatment of *non-H. pylori Helicobacteria* which are primarily infecting animals including preferably pets, preferably cats and dogs, and which also zoonotically infect human. Examples of zoonotic gastric pathogens includes *H. felis* and *H. heilmannii*. The present invention is not directed to binding specificities of *H. mustellae* included only as control which is not known to infect human or common pet animals such as cats and dogs.

Glycolipid and carbohydrate nomenclature is according to the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (Carbohydrate Res. 1998, 312, 167; Carbohydrate Res. 1997, 297, 1; Eur. J. Biochem. 1998, 257, 29).

It is assumed that Gal, Glc, GlcNAc, and Neu5Ac are of the D-configuration, Fuc of the L-configuration, and all the monosaccharide units in the pyranose form. Glucosamine is referred as GlcN or $GlcNH_2$ and galactosamine as GalN or $GalNH_2$. Glycosidic linkages are shown partly in shorter and partly in longer nomenclature, the linkages of the Neu5Ac-residues $\alpha 3$ and $\alpha 6$ mean the same as $\alpha 2$-3 and $\alpha 2$-6, respectively, and with other monosaccharide residues $\alpha 1$-3, $\beta 1$-3, $\beta 1$-4, and $\beta 1$-6 can be shortened as $\alpha 3$, $\beta 3$, $\beta 4$, and $\beta 6$, respectively. Lactosamine refers to N-acetyllactosamine, Galβ4GlcNAc, and sialic acid is N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc) or any other natural sialic acid. Term glycan means here broadly oligosaccharide or polysaccharide chains present in human or animal glycoconjugates, especially on glycolipids or glycoproteins. In the shorthand nomenclature for fatty acids and bases, the number before the colon refers to the carbon chain length and the number after the colon gives the total number of double bonds in the hydrocarbon chain. Abbreviation GSL refers to glycosphingolipid. Abbreviations or short names or symbols of glycosphingolipids are given in the text and Table 2. *zHelicobacter* refers also to the bacteria similar to *zHelicobacter*.

The expression "terminal oligosaccharide sequence" indicates that the oligosaccharide is not substituted to the non-reducing end terminal residue by another monosaccharide residue.

The term "α3/β3" indicates that the adjacent residues in an oligosaccharide sequence can be either α3- or β3- linked to each other.

EXAMPLES

Gastric species examined in the present study included, *Helicobacter mustelae* ferret isolates from the National Collection of Type Cultures (NCTC) and the Culture Collection of the University of Gothenberg (CCUG), NCTC 12198/CCUG 25175 (equivalent strains from different sources tested), CCUG 23950 and CCUG 23951, *Helicobacter felis* CCUG 28539 from a cat, in addition, *H. pylori* strains CCUG 17874, CCUG 17875 and a clinical isolate 119/95 were used. Enterohepatic helicobacters of animal origin were purchased from the CCUG including, *Helicobacter canis* CCUG 33835, *Helicobacter bilis* CCUG 38995, *Helicobacter hepaticus* CCUG 33637, and *Helicobacter fennelliae* (CCUG 18820).

Glycolipid binding assays

Binding of Helicobacter spp. to glycosphingolipids, both acid and non-acid fractions. Glycosphingolipids were isolated by standard procedures (Karlsson, 1987). The identity of the purified glycosphingolipids was confirmed by mass spectrometry (Samuelsson *et al.*, 1990), proton NMR spectroscopy (Koerner *et al.*, 1983) and degradation studies (Stellner *et al.*, 1973; Yang and Hakomori, 1971).

Mixtures of glycosphingolipids (40 μg/lane) or pure compounds (2 μg/lane) were subsequently separated using thin-layer chromatography (TLC) on glass- or aluminum-backed silica gel 60 HPTLC plates (Merck, Darmstadt, Germany), with chloroform/methanol/water (60:35:8, by volume) as solvent system. Chemical detection was accomplished by anisaldehyde (Waldi, 1962). *Helicobacter* spp. were subjected to ^{35}S-labeling (Ångström *et al.*, 1998) and suspended in PBS (10^8 CFU/ml) with a specific activity of approximately 1 cpm per 100 organisms. Binding of the labeled-bacteria to glycosphingolipids separated by TLC was achieved using a bacterial-overlay technique

coupled with autoradiography detection using XAR-5 x-ray films (Eastman Kodak, Rochester, NY) as previously described (Hansson *et al.*, 1985).

The carbohydrate binding specificities of zHelicobacter species

It has been established previously that both *H. pylori* and *H. mustelae* bind gangliotetraosylceramide binding was demonstrated for *H. felis*, *H. canis* and *H. hepaticus* and *H. bilis* (Table 1). Furthermore, in common with *H. pylori* we found that both gastric and enterohepatic *Helicobacter* spp. tested were capable of binding to lactotetraosylceramide, lactosylceramide with phytosphingosine and/or hydroxy fatty acids and isoglobotriaosylceramide. In contrast, binding to Le^b glycosphingolipid was only observed for *H. pylori* CCUG 17875 (Table 1).

The binding of certain *H. pylori* strains to slow-migrating gangliosides in the acid glycosphingolipid fraction of human granulocytes is sialic acid-dependent (Miller-Podraza *et al.*, 1999), and this fraction was therefore used as an indicator of sialic acid-recognition. Binding to this fraction was noted for *H. hepaticus* CCUG 33637 (exemplified in Fig. 1B. lane 1) and *H. pylori* CCUG 17874 and occasionally for *H. mustelae* CCUG 25715 (Table 1). Sialic acid binding capacity assayed by other substances is also present at least in species of *H. bilis*.

The ability of predominantly gastric and enterohepatic species of *Helicobacter* to glycosphingolipids is indicative of the use of host-carbohydrate binding by these species in their adhesion strategies.

The binding specificities of different helicobacteria may be indicative of the ability to colonize a specific niche with different receptors being expressed in the intestine and hepatobillary tree. In addition, different strategies may be useful at different times during infection due to changes in antigen expression by inflamed tissue (Mahdavi *et al.* 2002). From the present study it is apparent that strains of hepatobillary helicobacters namely *H. hepaticus* and *H. bilis* share common adhesion strategies with *H. pylori*. These types of hepatobiliary pathogens have ability to bind various glycoconjugates and even some sialylated structures.

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TABLE 1. Binding of ^{35}S-labeled *Helicobacter* species to glycosphingolipids on thin-layer chromatograms

Trivial name	Structure	Binding[a] of glycosphingolipids to:							
		H.pylori 17874	*H.pylori* 17875	*H. felis* 28539	*H.canis* 33835	*H.hepaticus* 33637	*H.mustelae* 25715	*H.mustelae* 23950 & 23951	*H. bilis* 38995
LacCer	Galβ 4Glcβ1Cer[b]	+	+	+	+	+	+	+	+
Isoglobotri	Galα3Galβ4Glcβ1Cer	+	+	+	+	+	+	+	+
GgO4	Galβ3GalNAcβ4Galβ4Glcβ1Cer	+	+	+	+	+	+	+	+
Le^a-5	Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer	-	-	-	-	-	-	-	-
Le^b-6	Fucα2Galβ3(Fucα4)GlcNAcβ3Galβ4Glcβ1Cer	-	+	-	-	-	-	-	-
Globotetra	GalNAcβ3Galα4Galβ4Glcβ1Cer	-	-	-	-	-	-	-	-
Lactotetra	Galβ3GlcNAcβ3Galβ4Glcβ1Cer	+	+	+	+	+	+	+	+
Acid glycosphingolipids of human granulocytes		+	-	-	-	+	+	-	-
NeuGc-nLc$_6$	NeuGcα3(Galβ4GlcNAcβ3)$_2$Galβ4Glcβ1Cer	+	+	+	+	+	+	+	+

[a] Binding is defined as follows: + denotes a significant darkening on the autoradiogram when 2 μg (or 40 μg in the case of the last sample) was applied to TLC plates whereas - denotes no binding.

[b] Ceramide composition (t18:0-h16:0-h24:0)

What we claim is

1. The use of a galactoseβ3/4 -based binding epitope for *zHelicobacter* species comprising an oligosaccharide sequence according to the formula:

$$[Gal\beta y]_p[Hex(NAc)_r\alpha z/\beta z]_sGal\beta x[Glc(NAc)_u]_v \qquad (I)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, and z is either linkage position 3 or 4, and Hex is either Gal or Glc ,

so that

when v is 1 and u is 0 then x is 4,

when v is 0 then s is 1 and preferably also p is 1,

when s is 0 the also p is 0 and v is 1,

when p is 1, and y=3, Hex is Galβ or Glcβ and r=1, or p is 1 and y=4 and Hex is Glcβ and r=1 so that the terminal Gal is β3- or β4- linked to GlcNAcβ or the terminal Gal is β3-linked to GalNAcβ),

when p is 0 and z is 4, then Hex is Galβ and r is 1 so that the terminal monosaccharide structure is GalNAcβ4, or p =0 and z=3 so that the terminal is Hex-NAc/Hexα/β3),

when there is nonreducing terminal Galβ3/4, this can be further substituted by SAα3/6, wherein SA is a sialic acid, preferably NeuNAc, N-acetylneuraminic acid,

for the manufacture of a medicament or a therapeutic composition for prophylaxis or treatment of an infection in the presence of *zHelicobacter*.

2. The use according to claim 1, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

lactosylceramide, lactosylceramide comprising hydroxyl fatty acids, lactosylceramide with modified carbon 3 of a galactose residue and isoglobotriaocylceramide

3. The use according to claim 1, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

Galβ3GalNAcβ4Galβ4Glc, Galβ3GalNAcβ4Gal, Galβ3GalNAc, GalNAcβ4Gal and GalNAcβ4Galβ4Glc

4. The use according to claim 1, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharides consisting of:

Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, and GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc

5. The use according to claim 1, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

Galβ3GlcNAcβ3Gal, Galβ3GlcNAcβ3Galβ4Glc, Galβ3GlcNAcβ3Galβ4GlcNAc, Galβ3GlcNAcβ3Galβ3GlcNAc GlcNAcβ3Galβ4GlcNAc, Galβ4GlcNAcβ3Gal, Galβ4GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4GlcNAc, GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc, and GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAc

6. The use according to claim 1, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharides consisting of:

Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, and GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc.

7. The use according to claim 1, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

NeuNAcα3Galβ, NeuNAcα6Galβ, NeuNAcα3Galβ4Glc, NeuNAcα3Galβ4GlcNAc, NeuNAcα6Galβ4GlcNAc, and NeuNAcα6Galβ4Glc

8. A therapeutical composition comprising a galactoseβ3/4 -based binding epitope or epitopes for *zHelicobacter* species comprising an oligosaccharide sequence according to the formula:

$$[Gal\beta y]_p[Hex(NAc)_r\alpha z/\beta z]_sGal\beta x[Glc(NAc)_u]_v \quad (I)$$

wherein p, r, s, u and v are each independently 0 or 1, and y is either linkage position 3 or 4, x is either linkage position 3 or 4, and z is either linkage position 3 or 4, and Hex is either Gal or Glc ,

so that

when v is 1 and u is 0 then x is 4,

when v is 0 then s is 1 and preferably also p is 1,

when s is 0 the also p is 0 and v is 1,

when p is 1, and y=3, Hex is Galβ or Glcβ and r=1, or p is 1 and y=4 and Hex is Glcβ and r=1 so that the terminal Gal is β3- or β4- linked to GlcNAcβ or the terminal Gal is β3-linked to GalNAcβ),

when p is 0 and z is 4, then Hex is Galβ and r is 1 so that the terminal monosaccharide structure is GalNAcβ4, or p =0 and z=3 so that the terminal is HexNAc/Hexα/β3),

when there is nonreducing terminal Galβ3/4, this can be further substituted by SAα3/6, wherein SA is a sialic acid, preferably NeuNAc, N-acetylneuraminic acid, preferably together with pharmaceutically acceptable carriers and adjuvants.

9. The pharmaceutical composition according to claim 8, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

lactosylceramide, lactosylceramide comprising hydroxyl fatty acids, lactosylceramide with modified carbon 3 of a galactose residue and isoglobotriaocylceramide

10. The pharmaceutical composition according to claim 8, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, and
GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc

11. The pharmaceutical composition according to claim 8, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, and
GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc

12. The pharmaceutical composition according to claim 8, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

Galβ3GlcNAcβ3Gal, Galβ3GlcNAcβ3Galβ4Glc,
Galβ3GlcNAcβ3Galβ4GlcNAc, Galβ3GlcNAcβ3Galβ3GlcNAc
GlcNAcβ3Galβ4GlcNAc, Galβ4GlcNAcβ3Gal, Galβ4GlcNAcβ3Galβ4Glc,
Galβ4GlcNAcβ3Galβ4GlcNAc, GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc, and
GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAc

13. The pharmaceutical composition according to claim 8, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

Galβ3GlcNAcβ3Galβ4Glc, Galβ4GlcNAcβ3Galβ4Glc, and
GlcNAcβ3Galβ4GlcNAcβ3Galβ4Glc.

14. The pharmaceutical composition according to claim 8, wherein the binding epitope for *zHelicobacter* is selected from the group of receptor oligosaccharide sequences consisting of:

NeuNAcα3Galβ, NeuNAcα6Galβ, NeuNAcα3Galβ4Glc, NeuNAcα3Galβ4GlcNAc, NeuNAcα6Galβ4GlcNAc, and NeuNAcα6Galβ4Glc

15. The composition according to claim 8, wherein the binding epitope for *zHelicobacter* is in monovalent form.

16. The composition according to claim 8, wherein at least one of said compounds is linked to a polyvalent carrier.

17. The composition according to claim 16, wherein said polyvalent carrier is a carbohydrate carrier or soluble carbohydrate carrier.

18. The composition according to claim 17, wherein said carbohydrate carrier is a bacterial polysaccharide or part of bacterial polysaccharide also comprising the receptor oligosaccharide sequence.

19. The composition according to any of the claims 8-18, for prophylaxis or treatment of gastrointestinal or hepatobiliary infection.

20. The composition according to claim 19, wherein said gastrointestinal infection causes diarrhea or inflammatory bowel disease.

21. The composition according to claim 19, wherein said infection causes a liver disease or liver cancer or gastric disease, gastric ulcers disease or gastric cancer.

22. The composition according to any one of claims 19-21, wherein said infection is caused by *zHelicobacteria*.

23. The composition according to any one of claims 8-22, for the treatment of a human patient.

24. The composition according to any one of claims 8-22, for the treatment of an animal patient, preferably a cat or dog.

25. A nutritional composition or a nutritional additive comprising at least one galactoseβ3/4 -based binding epitope for *zHelicobacter* species as defined in any of the claims 1-7 for prophylaxis or treatment as defined in any one of claims 19-24.

26. A nutritional composition or a nutritional additive according to claim 25 further comprising a probiotic microorganism or a prebiotic substance.

27. Use of a composition comprising a pathogen receptor as defined in any one of claims 1-7 as a part of filter material to purify pathogens from liquid food, beverages and water by filtering.

28. Use of a composition comprising a pathogen receptor as defined in any one of claims 1-7 in diagnostics of *zHelicobacter* binding to at least three oligosaccharide sequences as defined in any of the claims 1-7.

29. Use of a composition comprising pathogen receptors as defined in any of the claims 1-7 in diagnostics of a pathogen binding to at least four oligosaccharide sequences as defined in any of the claims 1-7.

30. A method of treatment for the conditions due to the presence of *zHelicobacter*, wherein a pharmaceutically effective amount of a binding epitope for zHelicobacter as defined in any one of claims 1-7 is administered to a subject in need of such treatment.

31. A soluble polyvalent substance comprising at least two oligosaccharide sequences from different groups defined in any one of the claims 1-7.

32. Infant formula comprising a galactoseβ3/4 -based binding epitope for *zHelicobacter* species as defined in any one of claims 1-7.

33. A food preservative, mouth hygiene product, topical, washing or cosmetic product comprising a galactoseβ3/4 -based binding epitope for *zHelicobacter* species as defined in any one of claims 1-7.

34. Use of a galactoseβ3/4 -based binding epitope or epitopes for *zHelicobacter* species as defined in any one of claims 1-7 for use *ex vivo*.

35. A composition comprising at least two substances as defined in any one of claims 1-7 for the manufacture of a medicament or a therapeutic composition for use according to any of the claims.

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(57) Abstract

The invention provides therapeutical substances comprising a pathogen-inhibiting oligosaccharide sequence for the manufacture of a medicament. The present invention especially describes an oligosaccharide-containing substance or epitope binding to enterohepatic zoonotic *Helicobacter* species, and use thereof in, e.g., pharmaceutical, nutritional and other compositions for prophylaxis and treatment of conditions due to the presence of zoonotic *Helicobacter*. The invention is also directed to the use of the receptors for diagnostics of zoonotic *Helicobacter* and consumer product uses.

FIG. 1